THERAPEUTIC MANIPULATION OF GUT MICROBIOTA

Antonio Gasbarrini, Francesca Ponziani
GUT Microbiota is implicated in the pathogenesis of all GI diseases

- Inflammatory Bowel Diseases
- Intestinal Bacterial Overgrowth
- Irritable Bowel Syndrome
- Diverticular disease
- Food Intolerance
- GI Cancer
- Liver diseases
- Bile tract diseases
- Pancreatic diseases
- Obesity and Metabolic Syndrome
GUT Microbiota modulation

- Is it possible to modulate it?
- How?
- Is it risky?
Quali-quantitative alterations of gastric, jejuno-ileal and colonic microbiota

GUT microbiota related diseases

DYSBIOSIS (overgrowth/reduction)

PROTECTIVE FACTORS

ADVERSE FACTORS

GUT MICROBIOTA MODULATION

GUT microbiota related diseases
**GUT MICROBIOTA MODULATION**

*Diet and Nutritional Support*

Caloric amount, minerals, vitamins..

Diet composition (fibers, fruits..)

*Removal of predisposing conditions*

Treat diabetes, endocrine, other motility disorders..

Surgery or prokinetics when indicated

Antiacid, immunosuppressants, drugs that affect

GUT motility...

*Drugs*

Biotherapy

Antibiotics
GUT MICROBIOTA MODULATION

**Diet and Nutritional Support**

- Caloric amount, minerals, vitamins
- Diet composition (fibers...)

**Removal of predisposing conditions**

- Treat diabetes, endocrine, other motility disorders..
- Surgery or prokinetics when indicated
- PPI/antiacid, immunosuppressants, other drugs that affect GUT motility..

**Drugs**

- Biotherapy
- Antibiotics
GUT MICROBIOTA MODULATION

Diet and Nutritional Support
Caloric amount, minerals, vitamins
Diet composition (fibers...)

Removal of predisposing conditions
Treat diabetes, endocrine, other motility disorders..
Surgery or prokinetics when indicated
Antiacid, immunosuppressants, drugs that affect GUT motility...

Drugs
Biotherapy
Antibiotics
Targets for GI antibiotic therapy

1. *Eradication of specific pathogens*

2. *Modulate mutualistic bacteria*
Antibiotic therapy in the clinical practice

- **Antibiotics are used to fight infections by pathogens**
- **Different antibiotic classes are used according to specific drug characteristics**
- **Antibiotic usage is at risk of side effects and clinical consequences**
- **Antibiotics have to be chosen according to specific objectives**
Targets for GI antibiotic therapy

1. Eradication of specific pathogens

2. Modulate commensal bacteria of gut microbiota
Antibiotic therapy to modulate «aspecifically» gut microbiota

- It is not clearly established which bacteria are reduced
- Risks of antibiotic usage are not clear
- Need of better knowledge of the GUT barrier in order to establish a new paradigm
A modulation of GUT Microbiota has been suggested in several GI diseases

- Inflammatory Bowel Diseases
- Intestinal Bacterial Overgrowth
- Irritable Bowel Syndrome
- Diverticular disease
- Food Intolerance
- GI Cancer
- Liver diseases
- Bile tract diseases
- Pancreatic diseases
- Obesity and Metabolic Syndrome
Strong rationale for a therapeutical modulation of GUT microbiota during progression of chronic liver diseases
GUT microbiota: a key player in hepatic encephalopathy

Moriwaki H et al J Gastr Hepatol 2009
Antimicrobial: Neomycin

Used for HE for the past 40 years

**PAUCITY OF CLINICAL DATA** to support the efficacy

**SIDE EFFECTS:** ototoxicity, neurotoxicity in renal insufficiency

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<table>
<thead>
<tr>
<th>Trial</th>
<th>Comparison (study design)</th>
<th>No. of patients</th>
<th>Treatment duration</th>
<th>Assessment</th>
<th>Overall efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strauss et al. [26]</td>
<td>Neomycin vs placebo (double-blind, randomized)</td>
<td>39</td>
<td>~7 days</td>
<td>Time to HE grade level change</td>
<td>Neomycin = placebo</td>
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<tr>
<td>Orandi et al. [27]</td>
<td>Neomycin vs lactulose (single-blind, randomized)</td>
<td>173</td>
<td>14 days</td>
<td>Mental status, asterixis score, EEG, ammonia levels, HE change</td>
<td>Neomycin = lactulose</td>
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<tr>
<td>Atterbury et al. [28]</td>
<td>Neomycin vs lactulose (double-blind, randomized)</td>
<td>35(^a)</td>
<td>~7 days</td>
<td>Mental status, asterixis score, EEG, ammonia levels, HE change</td>
<td>Neomycin = lactulose</td>
</tr>
<tr>
<td>Conn et al. [29]</td>
<td>Neomycin vs lactulose (double-blind, randomized, crossover)</td>
<td>29</td>
<td>10 days each arm before crossover</td>
<td>Mental status, asterixis score, EEG, ammonia levels, PSE index</td>
<td>Neomycin = lactulose</td>
</tr>
</tbody>
</table>

\(a\) 35 patients, 45 episodes of HE.

PSE = portal systemic encephalopathy.

*Paula V et al, Drugs 2010*
# Antimicrobial: Rifaximin

## Table IV. Comparison of rifaximin and disaccharides for hepatic encephalopathy (HE) treatment

<table>
<thead>
<tr>
<th>Trial</th>
<th>Comparison (study design)</th>
<th>No. of patients</th>
<th>Treatment duration</th>
<th>Assessment</th>
<th>Overall efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Festi et al. [30]</td>
<td>Lactulose (open-label)</td>
<td>21</td>
<td>21 days</td>
<td>Neurological signs of HE, asterixis score, HRNB, EEG, ammonia levels</td>
<td>Rifaximin = lactulose</td>
</tr>
<tr>
<td>Bucci and Palmier [31]</td>
<td>Lactulose (double-blind, double-dummy)</td>
<td>58</td>
<td>15 days</td>
<td>Neurological status, asterixis score, HRNB, cancellation tasks, EEG, ammonia levels</td>
<td>Rifaximin &gt; lactulose</td>
</tr>
<tr>
<td>Massa et al. [32]</td>
<td>Lactulose (double-blind, double-dummy)</td>
<td>40</td>
<td>15 days</td>
<td>HE index severity, mental status, cancellation tasks, HRNB, EEG</td>
<td>Rifaximin &gt; lactulose</td>
</tr>
<tr>
<td>Fera et al. [33]</td>
<td>Lactulose (double-blind, double-dummy)</td>
<td>40</td>
<td>First 2 weeks of each month × 3 months</td>
<td>Mental status, asterixis score, cancellation tasks, HRNB, EEG, ammonia levels, PSE index</td>
<td>Rifaximin &gt; lactulose</td>
</tr>
<tr>
<td>Mas et al. [34]</td>
<td>Lactitol (double-blind, double-dummy)</td>
<td>103</td>
<td>5–10 days</td>
<td>Mental status, asterixis score, EEG, ammonia levels, PSE index, psychometric tests</td>
<td>Rifaximin = lactitol</td>
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<tr>
<td>Leevy and Phillips [35]</td>
<td>Lactulose (crossover)</td>
<td>145</td>
<td>≥6 months lactulose ≥6 months rifaximin</td>
<td>HE grade, asterixis score</td>
<td>Rifaximin &gt; lactulose</td>
</tr>
<tr>
<td>Paik et al. [36]</td>
<td>Lactulose (open-label)</td>
<td>54</td>
<td>7 days</td>
<td>Ammonia levels, flapping tremor, mental status, HE index, psychometric tests</td>
<td>Rifaximin = lactulose</td>
</tr>
</tbody>
</table>

HRNB = Halstead-Reitan Neuropsychological Test Battery; PSE = portal systemic encephalopathy.

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*Paula V et al, Drugs 2010*  *Bajaj JS et al, Alim Pharm Ther 2010*
Rifaximin Treatment in Hepatic Encephalopathy

Nathan M. Bass, M.B., Ch.B., Ph.D., Kevin D. Mullen, M.D., Arun Sanyal, M.D., Fred Poordad, M.D., Guy Neff, M.D., Carroll B. Leevy, M.D.,* Samuel Sigal, M.D., Muhammad Y. Sheikh, M.D., Kimberly Beavers, M.D., Todd Frederick, M.D., Lewis Teperman, M.D., Donald Hillebrand, M.D., Shirley Huang, M.S., Kunal Merchant, Ph.D., Audrey Shaw, Ph.D., Enoch Bortey, Ph.D., and William P. Forbes, Pharm.D.

140 PTS: RIFAXIMIN 550 mg twice a day for 6 months

159 PTS: PLACEBO twice a day for 6 months

AIMs:

Primary: time to the first breakthrough episode of hepatic encephalopathy

Secondary: time to the first hospitalization involving hepatic encephalopathy
Primary end point:
**Rifaximin maintained remission from hepatic encephalopathy more effectively than did placebo for a 6-month periods**
- Hazard ratio 0.42
- Relative risk reduction 58%

Secondary end point:
**Rifaximin significantly reduced the risk of hospitalization involving hepatic encephalopathy**
- Hazard ratio 0.50
- Relative risk reduction 50%

*Bass et al. N Eng J Med 2010*
MINIMAL HEPATIC ENCEPHALOPATHY

COGNITIVE PROFILE which CANNOT BE DIAGNOSED CLINICALLY

- Psychomotor slowing, affect 70-75% of cirrhotics
- Cognitive deficits in visual–spatial perception, attention..
- Adversely affect daily activities such as driving ability, sociality..
- No correlation with seric ammonia

**DIAGNOSIS:** Neuropsychometric test (NP), NCT-A, NCT-B, digit symbol test (DST), PSE-syndrome test….

- Flicker frequency, inhibitory control test, cognitive drug research, RMN with diffusion coefficient
Role of small intestinal bacterial overgrowth and delayed gastrointestinal transit time in cirrhotic patients with minimal hepatic encephalopathy

Ankur Gupta², Radha K Dhiman¹,* , Savita Kumari², Satyavati Rana³, Ritesh Agarwal⁴, Ajay Duseja¹, Yogesh Chawla¹

➢ 26% of cirrhotics with MHE had SIBO

➢ SIBO prevalence: CHILD A 31%
               CHILD B-C 70%

➢ OCTTT significantly prolonged in SIBO + (175 vs 127 min, p <0.0001)

Gupta A et al, J Hepatology 2010
The total SIP score improved significantly only in rifaximin group (baseline: 11.67 vs 8 weeks, 6.45)

Improvement in HRQOL correlated with improvement in NP tests

Sandeep et al, Am J Gastroent 2011
MHE patients have an increased risk of driving offenses and have poor insight into their driving skills.

RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROL with RIFAXIMIN 1100 MG x 8 weeks

Primary end point: evaluate the effect of driving performance using a driving simulator

Secondary outcomes: cognitive performance, quality of life, and change in the systemic inflammatory milieu and neuroglial function markers

Bajaj JS et al, Gastroenterology 2011
RIFAXIMIN showed an higher rate of improvement in total driving errors, specifically speeding tickets and navigation of illegal turns on a driving simulator compared with those on placebo. This was accompanied by improved cognitive performance and psychosocial aspects of quality of life.

**Bajaj JS et al, Gastroenterology 2011**
Randomised clinical trial: rifaximin improves health-related quality of life in cirrhotic patients with hepatic encephalopathy - a double-blind placebo-controlled study


219 cirrhotics in remission from HE (Conn score 0 or 1) and a documented history of recurrent HE episodes

Rifaximin 550 mg x 2 (n 101)

Placebo (n 118)

for 6 months

Sanyal A et al. Aliment Pharm Ther 2011
Chronic Liver Disease Questionnaire Domain Score:
Rifaximin vs Placebo
A modulation of GUT Microbiota has been suggested in several GI diseases

- Inflammatory Bowel Diseases
- Intestinal Bacterial Overgrowth
- Irritable Bowel Syndrome
- Diverticular disease
- Food Intolerance
- GI Cancer
- Liver diseases
- Bile tract diseases
- Pancreatic diseases
- Obesity and Metabolic Syndrome
142 pts affected by SIBO (glucose BT) were randomized to:

- Rifaximin 1200 mg/d for 7 days
- Metronidazole 750 mg/d for 7 days

Group 1: 63%
Group 2: 43%

* p<0.05

Lauritano and Gasbarrini, Eur Rev Med Pharm 2009
90 pts affected by SIBO (glucose BT) were randomized to a 7-day therapy with:

1. Rifaximin 600 mg/day
2. Rifaximin 800 mg/day
3. Rifaximin 1200 mg/day

Group 1: 17%
Group 2: 27%
Group 3: 60%

* p<0.05
GI symptoms (abdominal pain and diarrhoea) in IBS and after eradication of bacterial overgrowth with an antibiotic therapy

**Pittfalls:**
1. **SIBO diagnosis with LBT**
2. **Different antibiotics:**
   - Neomycin
   - Ciprofloxacin
   - Metronidazole
   - Doxicicline
3. **Lack of control group**

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**Table 3. Comparison of Rome Criteria-Positive Subjects Before and After Treatment With Successful and Unsuccessful Eradication of SIBO**

<table>
<thead>
<tr>
<th></th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>Total</th>
<th>$\chi^2$</th>
<th>$p$</th>
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<tbody>
<tr>
<td>Not eradicated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rome +</td>
<td>22</td>
<td>18</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rome −</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total eradicated</td>
<td>22</td>
<td>22</td>
<td>44</td>
<td>4.0</td>
<td>0.05</td>
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<tr>
<td>Rome +</td>
<td>25</td>
<td>13</td>
<td>38</td>
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<td></td>
</tr>
<tr>
<td>Rome −</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>12.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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*Pimentel – Am J Gastroenterol 2000*
Antibiotics in IBS: Neomycin

- Neomycin improves GI symptoms in IBS (placebo control)
- Clinical response correlates with LBT normalization

PITTFALLS
- SIBO diagnosis with LBT
- LBT normalization only in 20% pts:
  - Antibiotics resistance?
  - Inadequate antibiotics duration and/or dosage?

Figure 3. Comparison of percent reported bowel normalization between and within gender groups.

Pimentel – Am J Gastroenterol 2003
Neomycin effect on CH4 production

- IBS-C is associated with overproduction CH4
- Neomycin reduces CH4 production and improves GI symptoms in IBS-C pts

Pimentel – Am J Gastroenterol 2003
Pimentel – Dig Dis Sci 2006
Antibiotics in IBS: Rifaximin

- Double-blind, randomized, controlled study
- 87 pz single center study
- Clinical diary of symptoms up to 10 weeks after end of therapy

- Rifaximin improves global symptoms and bloating score in IBS patients

*Pimentel et al, Ann Intern Med 2006*
Rifaximin Therapy for Patients with Irritable Bowel Syndrome without Constipation

Mark Pimentel, M.D., Anthony Lembo, M.D., William D. Chey, M.D.,
Salam Zakko, M.D., Yehuda Ringel, M.D., Jing Yu, Ph.D.,
Shadreck M. Mareya, Ph.D., Audrey L. Shaw, Ph.D., Enoch Bortey, Ph.D.,
and William P. Forbes, Pharm.D., for the TARGET Study Group*

14-Day double-blind treatment phase (rifaximin 550 mg 3 times daily or placebo)

10-Wk follow-up (no study medication)

Randomization 1:1

Primary efficacy evaluation period

- Efficacy
  - Weekly global IBS symptoms
  - Weekly IBS-related bloating
  - Daily IBS symptoms
  - Quality of life
- Safety
  - Adverse effects and concomitant medications
  - Vital signs
  - Laboratory tests
  - Physical examinations

Day

1  7  14  21  28  35  42  49  56  63  70  77  84

End of study
Adequate relief was defined as self-reported relief from symptoms for at least 1 week of every 2-week period.
Meta-analysis of rifaximin in the management of uncomplicated **Diverticular Disease**: *symptoms relief*

**Figure 2** | Rate differences (RD) (95% CI) for complete symptom relief at the end the follow-up in prospective randomised trials addressing Rifaximin group vs. control group. Random effect model.
Long term cyclic administration of the poorly absorbed antibiotic Rifaximin in symptomatic, uncomplicated colonic diverticular disease

Colecchia et al, World J. Gastroenterol, 2007
BMJ Clinical Evidence
Recommendation

Rifaximin plus a dietary fibre supplement (glucomannan) may be more effective at 12 months at relieving symptoms of uncomplicated diverticular disease compared with dietary fibre supplementation alone.

Humes et al, BMJ Clinical Evidence 2011
Antibiotics to modulate gut microbiota

Many pitfalls remain present:

- **Diagnostic criteria**
- **Study design**
- Lack of placebo
- Underpowered study
- Underlying Intestinal Bacterial Overgrowth/Dysbiosis
- Role of enterotypes
- Risk of gut microbiota regrowth
- Different antibiotics
- Different dosage and duration
- Risk of antibiotic usage
H2 area under the curve in IBS vs HV

57 IBS-D patients and 29 healthy volunteers (HV) were enrolled. H2-lactulose AUC was significantly higher in IBS-D pts than HV (3449 ± 368 vs 2133 ± 38 ppm/min, p <0.001).

Scarpellini et al, UEGW 2010
**H2 AUC in IBS-B+ vs IBS-B-**

- A statistical difference between IBS-D pts with vs without bloating (4397 ± 619 vs 2501 ± 180 ppm/min, p < 0.05) but not between non-bloating-IBS pts and HV (2501 ± 180 vs 2133 ± 38 ppm/min, p = NS) was observed.

- Moreover there was a statistical correlation between bloating VAS AUC and H2 AUC values in bloating- but not in non-bloating-prevalent IBS pts (R=0.33, p=0.005; R=0.08, p=NS, respectively).

* Scarpellini et al, UEGW 2010

![Graph showing the comparison of AUC between different groups](attachment:image.png)
Many pitfalls remain present:

- Diagnostic criteria
- Study design
- Lack of placebo
- Underpowered study
- Underlying Intestinal Bacterial Overgrowth/Dysbiosis
- Role of gut barrier, other microbiota components, enterotypes
Bacteria are one of the components of the GUT barrier.
Candida overgrowth is a consequence of disturbances in the host’s defense systems:

- Antibiotic therapy
- Change in:
  - physiological gut microbiota
  - pH
  - partial CO2 pressure
  - amino acid availability
  - iron deficiency

... 

Thewes S, Mol Microbiol 2007
Gut microbiota functions

- Barrier effect
- Immuno-stimulation/tolerance
- Synthesis
- Metabolic/trophic functions
- Drug metabolism
- Toxin metabolism
- Behavior conditioning

...specific effects in each GI tract!
BACTERIAL DIVERSITY

1 kg of bacteria, >3,300,000 genes

8 bacterial divisions (superkingdoms)
95% genes identity

>1100 species
Over 99% genes identity

>15000 strains
100% genes identity

Microbiome ↔ Metabolome

Leser et al, Environ Microbiol 2011
Qin et al, Nature 2011
PYRAMID OF LIFE: human gut microbiota

- >3.000.000 Genes
- >58.000 Enzymes
- >25.000 Chemicals

Genomics → Proteomics → Metabolomics

Kau et al, Nature 2011
Qin et al, Nature 2011
PHILOGENETIC DIFFERENCES BETWEEN ENTEROTYPES

3 enterotypes identifiable by the variation in the levels of one of three genera:

ENTEROTYPE 1: Bacteroides
ENTEROTYPE 2: Prevotella
ENTEROTYPE 3: Ruminococcus

Arumugam – Nature 2011
MANY PITFALLS REMAIN PRESENT:

- Diagnostic criteria
- Study design
- Lack of placebo
- Underpowered study
- Underlying Intestinal Bacterial Overgrowth/Dysbiosis
- Role of other microbiota components and enterotypes
- Gut microbiota regrowth
Risk of recurrence of INTESTINAL BACTERIAL OVERGROWTH?

- Nutritional support
- Removal of predisposing conditions
- Modulation of contaminating flora
  - Biotherapy
  - Antibiotics (Systemic, Topic)

..High recontamination: >25% after 3-month
>75% after 12-month

Lauritano EC, Gasbarrini A et al, Am J Gastro 2008
RECURRENT OF INTESTINAL BACTERIAL OVERGROWTH AFTER ANTIBIOTICS

Pts (N=61)  3 months  6 months  9 months

Recurrence rate %
(I.C. 95%)

13.1 (4.6-21.6)
27.9 (16.6-39.2)
42.6 (30.2-55.0)

Lauritano EC, Gasbarrini A et al, Am J Gastro 2008
Table 2. Changes in fecal bacterial population after oral rifaximin administration in healthy volunteers (from Testa et al. [63])

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Weeks</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>•10^8</td>
</tr>
<tr>
<td>Other enterobacters</td>
<td>•10^7</td>
</tr>
<tr>
<td>Enterococci</td>
<td>•10^7</td>
</tr>
<tr>
<td><em>Bacteroides</em> spp.</td>
<td>•10^9</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>•10^8</td>
</tr>
<tr>
<td>Anaerobic cocci</td>
<td>•10^7</td>
</tr>
</tbody>
</table>

Rifaximin (800 mg) was given in two daily doses for 5 days after the first stool collection.
Antibiotics in to modulate microbiota

MANY PITTFALLS REMAIN PRESENT:

- Diagnostic criteria
- Study design
- Lack of placebo
- Underpowered study
- Underlying Intestinal Bacterial Overgrowth/Dysbiosis
- Role of other microbiota components/enterotypes
- Risk of gut microbiota regrowth
- Different antibiotics
- Different dosage and duration
- Risk of antibiotic usage
## Antibiotics demodulate GUT microbiota

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose mg/day</th>
<th>Days of administration</th>
<th>Number of administration patients</th>
<th>Impact on</th>
<th>Emergence of resistance</th>
<th>Overgrowth</th>
<th>Concentration range in faeces (mg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic G+cocci bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Phenoxymethylpenicillin</td>
<td>1000x2</td>
<td>10</td>
<td>10</td>
<td>↓</td>
<td></td>
<td></td>
<td>&lt;D</td>
<td>4</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>500x3</td>
<td>7</td>
<td>10</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>NE</td>
<td>6</td>
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<td>Amoxicillin</td>
<td>250x3</td>
<td>7</td>
<td>38</td>
<td>↓</td>
<td>↑</td>
<td></td>
<td>NE</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>500x3</td>
<td>7</td>
<td>10</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>NE</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>500x3</td>
<td>7</td>
<td>40</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>NE</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1000x2</td>
<td>14</td>
<td>14</td>
<td>↑</td>
<td>↓</td>
<td></td>
<td>NE</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1000x2</td>
<td>14</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td></td>
<td>NE</td>
<td>11</td>
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<tr>
<td>Amoxicillin/</td>
<td>875/125*</td>
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<td>12</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>&lt;D</td>
<td>12</td>
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<tr>
<td>clavulanic acid</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Piperacillin/ tazobactam</td>
<td>4000/20</td>
<td>4-8</td>
<td>20</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>&lt;D</td>
<td>13</td>
</tr>
<tr>
<td>Pymecillinamin</td>
<td>400x2</td>
<td>7</td>
<td>15</td>
<td>↓</td>
<td>↑</td>
<td></td>
<td>&lt;D</td>
<td>15</td>
</tr>
</tbody>
</table>

↓ = mild to moderate suppression 2-4 log10 CFU/g faeces, ↑ = increase in number of microorganisms during therapy, = = no significant change, NE = not examined, D = the detection limit, * = ratio of the involved preparations.

_Sullivan A, Lancet ID 2001_
## Antibiotics demodulate GUT microbiota

<table>
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<tr>
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<th>Days of administration</th>
<th>Impact on</th>
<th>Emergence of resistance</th>
<th>Overgrowth</th>
<th>Concentration range or mean (SD) in faeces (mg/kg)</th>
<th>Reference</th>
</tr>
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<td>↑↑</td>
<td>1·3–10·3</td>
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<td>↓↓ G+ cocci NE</td>
<td>↓↓</td>
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\(↓↓\)=strong suppression \(>4\ \log_{10}\text{CFU/g faeces}\), \(↓\)=mild to moderate suppression \(2–4\ \log_{10}\text{CFU/g faeces}\), \(↑\)=increase in number of microorganisms during therapy, \(→\)=no significant change, \(⇒\)=major impact, \(⇒\)=minor impact, \(NE\)=not examined, \(D\)=detection limit, \(∗\)=dose-dependent impact on the flora.
## Antibiotics associated Diarrhea

<table>
<thead>
<tr>
<th>Author (ref)</th>
<th>Place</th>
<th>Study type</th>
<th>Definition of AAD</th>
<th>Prevalence of AAD (%)</th>
<th>Age group</th>
<th>All or particular antibiotics</th>
<th>Outpatients or inpatients</th>
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<tr>
<td>Mitchell, et al. (9)</td>
<td>USA</td>
<td>Prevalence</td>
<td>adequate</td>
<td>22/76 (28.9)</td>
<td>12-47 m</td>
<td>amoxicillin/ clavunate</td>
<td>outpatients</td>
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<tr>
<td>Vanderhoof, et al. (4)</td>
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<td>RCT*</td>
<td>adequate</td>
<td>25/95 (26)</td>
<td>6 m 10 y</td>
<td>all</td>
<td>outpatients</td>
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<tr>
<td>Arvola, et al. (5)</td>
<td>Finland</td>
<td>RCT</td>
<td>adequate</td>
<td>9/58 (16)</td>
<td>2 weeks to 12.8 years</td>
<td>all, but 38 of 58 received amoxicillin</td>
<td>outpatients</td>
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<td>Jirapinyo, et al. (6)</td>
<td>Thailand</td>
<td>CT**</td>
<td>not known</td>
<td>8/10 (80)</td>
<td>1-36 m</td>
<td>all</td>
<td>inpatients</td>
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<tr>
<td>Turke, et al. (3)</td>
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<td>71/650 (11)</td>
<td>1 m-15.4 y</td>
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<td>outpatients</td>
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<td>La Rosa, et al. (7)</td>
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<td>CT</td>
<td>inadequate</td>
<td>31/50 (62)</td>
<td>Mean age 6.6 y</td>
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<td>Sekhi H, et al. (8)</td>
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<td>not known</td>
<td>16/27 (59)</td>
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<td>adequate</td>
<td>22/127 (17.3)</td>
<td>5 m-15 y</td>
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<td>both</td>
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<td>Damrongmanee and Karapol (2)</td>
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<td>adequate</td>
<td>14/225 (6.2)</td>
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<td>Poland</td>
<td>RCT</td>
<td>adequate</td>
<td>20/120 (17)</td>
<td>3 m-14 y</td>
<td>all</td>
<td>both</td>
</tr>
</tbody>
</table>

*RCT: Randomized controlled trial, **CT: Clinical Trial.
GUT microbiota-antibiotic resistance
Biochemistry of Antibiotic resistance

- Mutational alteration of the target protein
- Enzymatic inactivation of the drug
- Acquisition of genes for less susceptible target proteins from other species
- Bypassing of the target
- Preventing drug access to the target

GUT microbiota is at high risk of developing Antibiotic resistance: 1. Innovation Gap

✓ Between 1962 and 2000, no major classes of antibiotics were introduced

Fischbach M, Science 2009
Hormesis is the property of a compound that is beneficial at low concentrations and toxic at high concentrations

At low concentrations, antibiotics can trigger the expression of a specific set of genes that may be beneficial to bacteria; at higher concentrations, stress responses are induced and bacterial growth is inhibited

Martinez JL, FEMS Microbiol Rev 2009
Antibiotics usage specifically affects emergence of resistance in GUT microbiota

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
<th>Days of</th>
<th>Number of</th>
<th>Impact on</th>
<th>Emergence of resistance</th>
<th>Overgrowth</th>
<th>Concentration range in faeces (mg/kg)</th>
<th>Reference</th>
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<tr>
<td></td>
<td>mg/day</td>
<td>admin-</td>
<td>patients</td>
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<td>10</td>
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<td>7</td>
<td>15</td>
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↓ = mild to moderate suppression 2–4 log10 CFU/g faeces, ↑ = increase in number of microorganisms during therapy, = = no significant change, NE = not examined, D = the detection limit, *ratio of the involved preparations.

Antibiotics usage specifically affects emergence of resistance in GUT microbiota

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg/day)</th>
<th>Days of administration</th>
<th>Number of patients</th>
<th>Impact on Aerobic G+ cocci</th>
<th>Enterococci</th>
<th>Anaerobic Enterobacteria</th>
<th>Emergence of resistance</th>
<th>Overgrowth</th>
<th>Concentration range or mean (SD) in faeces (mg/kg)</th>
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<td>5</td>
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<td>NE</td>
<td>NE</td>
<td>↓↓</td>
<td>NE</td>
<td>1.1–9.3</td>
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<td>3</td>
<td>17</td>
<td>↑</td>
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<td>↓</td>
<td>NE</td>
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<td>↓</td>
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<td>↓</td>
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<td>↓</td>
<td>↓</td>
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<td>6</td>
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<td>↓</td>
<td>↓</td>
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<td>↓↓</td>
<td>↓</td>
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<td>↓</td>
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</tbody>
</table>

↓↓=strong suppression >1 log10 CFU/g faeces, ↓=mild to moderate suppression 2–4 log10 CFU/g faeces, ↑=increase in number of microorganisms during therapy, NE=no significant change, ±=major impact, (+)=minor impact, NE=not examined, D=detection limit, *dose-dependent impact on the flora.

After the onset of treatment, increase in resistant bacteria due to:
• resistant bacteria (purple rods), present at low levels, which increase in number
• horizontal gene transfer or mutation events (white arrow).
Some bacteria may be protected from antibiotic exposure in the mucin layer (yellow shading). A temporary decrease in diversity can also be seen.
Risk of antibiotic-resistance transfer?
Genetics of Antibiotic resistance

✓ Chromosomal resistance: vertically trasmissible by bacterial division

No risk of antibiotic-resistance transfer

Courvalin P, DLD 2006
Genetics of Antibiotic resistance

✓ Plasmid resistance: horizontally trasmissible by bacterial conjugation

High risk of antibiotic-resistance transfer

Courvalin P, DLD 2006
**GUT Anaerobes and antibiotic resistance**

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Antibiotic</th>
<th>Gene designation</th>
<th>Transferable</th>
<th>Transfer factor</th>
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<td>ermF, ermS</td>
<td>+</td>
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<td>Tetracycline</td>
<td>tetQ, tetX</td>
<td>+</td>
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<td>Cephalosporin</td>
<td>cepA, cbiA</td>
<td>ND†</td>
<td>Plasmid</td>
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<tr>
<td></td>
<td>Cefoxitin</td>
<td>cfxA</td>
<td>+</td>
<td>Transposon</td>
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<td>Metronidazole</td>
<td>nimA, nimB, nimC, nimD, nimE, nimF</td>
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<td>Quinolones</td>
<td>gyrA, gyrB, parC, parE</td>
<td>??</td>
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<td>Streptomycin</td>
<td>aadS*</td>
<td>+</td>
<td>Transposon</td>
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<td>catQ§, catP</td>
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<td>Clindamycin</td>
<td>ermQ, ermP</td>
<td>ND</td>
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<tr>
<td></td>
<td>Chloramphenicol</td>
<td>catD</td>
<td>+</td>
<td>Transposon</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
<td>ermZ, ermBZ</td>
<td>+</td>
<td>Transposon</td>
</tr>
<tr>
<td><strong>Clostridium butyricum</strong></td>
<td>Chloramphenicol</td>
<td>catA, catB</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td><strong>Prevotella spp</strong></td>
<td>Tetracycline</td>
<td>tetQ, tetO, tetM</td>
<td>+</td>
<td>Transposon (tetQ)</td>
</tr>
<tr>
<td><strong>Fusobacterium spp</strong></td>
<td>Tetracycline</td>
<td>tetM</td>
<td>+</td>
<td>Transposon</td>
</tr>
</tbody>
</table>

*Cryptic.
†Not determined.
‡A plasmid-borne imipenem resistance determinant has been isolated, but the gene has not been characterized.
§catQ was characterized from a non-conjugative strain.
*The exact nature of the transfer factor is unknown.

Vedantam G, Curr Opin in Microbiol 2003
Antibiotics effect on GUT microbiota

Resistant clone types
Number of clone types

Placebo
Clindamycin

Number of Bacteroides sp. clone types and percent of clones highly resistant to clindamycin x 24 months after 1 week of therapy

Jernberg C, ISME J 2007
A Rifaximin-resistance in the Gut Microbiota?

Fig. 4. Disappearance of rifaximin-resistant bacteria from human intestine after stopping the antibiotic treatment (week 0) (from De Leo et al. [65]).

Scarpignato et al, Digestion 2006
Antibiotics are often used in the clinical practice. As regards GI tract they can be used to:

1. Treat enteric infections
2. Modulate mutualistic GI bacteria

The topic antibiotic Rifaximin seem to be safe and effective for GI microbiota modulation.

Their usage affects deeply the GUT microbiota, leading to:

1. GI side effects (diarrhea, bloating…)
2. Risk of development of Antibiotic resistance